

We Claim:

1. A method of mutating a target DNA sequence of a plant comprising:

a. electroporating into a microspore of the plant a recombinagenic oligonucleobase that contains a first homologous region that has a sequence identical to the sequence of at least 6 base pairs of a first fragment of the target DNA sequence and a second homologous region which has a sequence identical to the sequence of at least 6 base pairs of a second fragment of the target DNA sequence, and an intervening region which contains at least 1 nucleobase heterologous to the target DNA sequence, which intervening region connects the first homologous region and the second homologous region;

10 a. culturing the microspore to produce an embryo; and

c. producing from the embryo a plant having a mutation located between the first and second fragments of the target DNA sequence.

2. The method of claim 1, wherein the recombinagenic oligonucleobase is an MDON and each of the homologous regions contains an RNA segment of at least 6 RNA-type nucleotides.

3. The method of claim 2, wherein the intervening region is at least 3 nucleotides in length.

4. The method of claim 2, wherein the first RNA segment contains at least 8 contiguous 2'-substituted ribonucleotides.

20 5. The method of claim 4 wherein the second RNA segment contains at least 8 contiguous 2'-substituted ribonucleotides.

6. The method of claim 2, wherein the sequence of the mutated target DNA sequence is homologous with the sequence of the MDON.

7. The method of claim 2, wherein the target DNA sequence is a first ALS DNA sequence, a second ALS gene, a psbA gene, a threonine dehydratase gene, a dihydrodipicolinate synthase gene, or an S14/rp59 gene

8. The method of claim 9, wherein the plant is a member of the family Brassicaceae.

9. The method of claim 9, wherein the plant is selected from the group consisting of *Brassica napus*, *Brassica rapa*, *Brassica oleracea*, and *Brassica juncea*.

10. The method of claim 2, wherein the target DNA sequence is selected from the group consisting of the genes encoding acetolactate synthase, green fluorescent protein, phosphoribosylanthranilate transferase, fatty acid desaturase, putrescine N-methyltransferase, acid invertase, UDP-glucose pyrophosphorylase, polyphenol oxidase,
5 O-methyl transferase, cinnamyl alcohol dehydrogenase, *etr-1* or a homolog thereof, ACC synthase, ACC oxidase, EPSP synthase, and protoporphopyrogen oxidase.

11. The method of claim 2, which further comprises making seeds from the plant or from progeny of the plant.

12. A method of making a localized, non-selectable mutation in a target DNA
10 sequence of a plant comprising the steps of:

a. introducing into a population of microspores of the plant a mixture comprising a first recombinagenic oligonucleobase and a second recombinagenic oligonucleobase wherein (i) the first recombinagenic oligonucleobase contains a first
15 homologous region which has a sequence identical to the sequence of at least 6 base pairs of a first fragment of a first target DNA sequence and a second homologous region which has a sequence identical to the sequence of at least 6 base pairs of a second fragment of the first target DNA sequence, and an intervening region which contains at least 1 nucleobase heterologous to the target DNA sequence, which intervening region connects the first homologous region and the second homologous region, and (ii) the second
20 recombinagenic oligonucleobase contains a first homologous region which has a sequence identical to the sequence of at least 6 base pairs of a first fragment of a second target DNA sequence and a second homologous region which has a sequence identical to the sequence of at least 6 base pairs of a second fragment of the second target DNA sequence, and an intervening region which contains at least 1 nucleobase heterologous to the target DNA
25 sequence, which intervening region connects the first homologous region and the second homologous region;

b. selecting microspores from the population having a selectable mutation located between the first and the second fragments of the first target DNA sequence from the population; and

30 c. identifying a selected microspore having a non-selectable mutation located between the first fragment and the second fragment of the second target DNA sequence.

13. The method of claim 12 further comprising culturing the microspore having a non-selectable mutation located between the first fragment and the second fragment of the second target DNA sequence to produce an embryo, and producing from the embryo a plant.
- 5 14. A plant or seed having a mutation in a DNA sequence that is in its wild-type genetic position, which DNA sequence is selected from the group consisting of the genes encoding acid invertase, UDP-glucose pyrophosphorylase, polyphenol oxidase, O-methyl transferase, cinnamyl alcohol dehydrogenase, ACC synthase, ACC oxidase, *etr-1*, homologs of *etr-1*, EPSP synthase, and protoporphopyrogen oxidase, and the sequence of
10 the genomic DNA within 23 KB of the mutation is the sequence of the wild type DNA, and the point mutation forms a stop codon or is a frameshift mutation.
15. The plant or seed of claim 14, in which the mutation forms a stop codon.
16. The plant or seed of claim 14 in which the sequence of genomic DNA within 40 KB of the selectable mutation is the sequence of the wild type DNA.
- 15 17. The plant or seed of claim 16 in which the sequence of genomic DNA within 100 KB of the selectable mutation is the sequence of the wild-type DNA.
18. The plant or seed of claim 14 in which the point mutation is a single basepair mutation.
19. The plant or seed of claim 14, further having a selectable point mutation in a
20 second DNA sequence and the sequence of genomic DNA within 23 KB of the selectable point mutation is the sequence of the wild type DNA.
20. The plant or seed of claim 14, in which the point mutation is a frameshift mutation.
21. The plant or seed of claim 14, in which the point mutation is a single base pair mutation.
- 25 22. A method of altering at least one base of a target DNA sequence of a plant comprising:
- a. providing a microspore of the plant;
 - b. introducing a mixed duplex oligonucleotide into the microspore;
 - c. producing a plant from the microspore having an alteration in at least one

base in a target DNA sequence caused by the mixed duplex oligonucleotide.

23. The method of claim 22 wherein the mixed duplex oligonucleotide is introduced into the microspore by electroporation.

5 24. A plant microspore comprising a mixed duplex oligonucleotide.

25. A composition of matter comprising a plurality of plant microspores and an aqueous solution comprising a mixed duplex oligonucleotide that is suitable for electroporation of the mixed duplex oligonucleotide into the microspores.

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